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Warm temperatures at bloom reduce fruit set in sweet cherry

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Summary

Warm springs have often been assumed as a prelude of a good fruit set in temperate fruit tree species. However, recently, evidences have accumulated on erratic fruit set under apparently good and warm springs in Mediterranean conditions. The fact that these observations mainly occurred in sweet cherry (Prunus avium), a species adapted to high latitudes and cold climates raised the question of whether warm temperatures at flowering could have a detrimental effect on fruit set. To evaluate this hypothesis two different sweet cherry cultivars were subjected under field conditions to a slight increase in temperature at bloom over two different years. While the minimum temperature remained stable, the maximum temperature increased 5-7ºC, resulting in a moderate increase of the average temperature of 1-3ºC. This was sufficient to drastically reduce fruit set in both years and cultivars. To know the vulnerable phase to warm temperatures the process was timed back: final fruit set differences were established in the first three weeks following pollination, but the onset of fruiting –when these differences appeared- was tracked back to one week after pollination. The process from pollination to fertilization was examined under both conditions. Fertilization occurred six days after pollination. Higher temperatures accelerated pollen tube growth rate but also reduced the number of growing pollen tubes along the style. In the ovary, the warm treatment accelerated ovule degeneration. These findings alert on the potential negative effect of even slight increases in temperature during cherry blooming, which nowadays -due to global warming trends- is a plausible and realistic scenario under Mediterranean climatic conditions.

Introduction

Climatic conditions at flowering are known to have an effect on the subsequent fruit set and cold and rainy springs may jeopardize pollination. Likewise cold temperatures at flowering reduce the speed of pollen tube growth and shorten the effective pollination period (Sanzol and Herrero, 2001). However, relatively warm springs have often been assumed as a prelude of a good fruit set in several temperate fruit tree species. Nevertheless, in the last years casuistry has accumulated on erratic fruit set in fruit tree species under apparently "good" and warm springs in Mediterranean regions. The fact that these observations mainly occurred in sweet cherry (Prunus avium L.), a species adapted to high latitudes and cold climates raised the question of whether warm temperatures at flowering could have a detrimental effect on the subsequent fruit set. In fact, the reproductive phase is one of the most sensitive plant developmental stages to heat stress (Hall, 1992) and both high and low temperatures at blooming time are known to have a detrimental effect on subsequent fruit set.

Different works have studied the effect of high temperatures on fruit set by applying temperature stress during the whole reproductive cycle in a range of different species (Monterroso and Wien, 1990; Hall, 1992; Gross and Kigel, 1994; Peet et al., 1998; Prasad et al., 2002, 2003). Although the results obtained have revealed a negative effect on fruit set, such experimental procedures could not disentangle the relative importance of the different developmental stages occurring from the flower to the whole plant levels.

Alternatively, the effect of temperature on particular processes has been evaluated. High temperature, alone or in combination with other environmental stresses, has been shown to affect both pollen and pistil functions (Erickson and Markhart, 2002, Young et al., 2004, Koti et al., 2005). However the sensitive stages seem to be species specific and depended on the type of stress. In sweet cherry, previous works have revealed a negative effect of high temperatures on the length of stigmatic receptivity (Hedhly et al., 2003) and ovule viability (Postweiler et al., 1985). Temperature also affects pollen tube kinetics and population census along the style (Hedhly et al., 2004) with a strong genotype-temperature interaction that could reflect the geographic origin of the pollen donor and its adaptation to the prevailing environmental conditions (Hedhly et al., 2005a).

However, field results and laboratory work are not always easy to merge. The main objective of this work is to evaluate whether moderately high temperatures at bloom affect fruit set in sweet cherry, and to characterize its effect on the reproductive phase. For this purpose, we applied an increase of temperature at flowering time for the first two weeks after anthesis under orchard conditions. We characterized the pattern of flower and fruit drop, ovary and

ovule growth following pollination, and the progamic phase spanning from pollination to fertilization.

Materials and methods

Plant material and experimental procedure

The experiments were carried out in a sweet cherry collection located at the Campus of Aula Dei in Zaragoza, Spain. The cultivars used were 'Vignola' and 'Sunburst' as pistil donors, and 'Napoleon' and 'Burlat' as pollen donors. The trees were 12 years old at the beginning of the trials, grafted on 'Santa Lucia 64' rootstocks, pruned as open center and with a plantation frame of 5 x 4 m. The increase of temperature in the warm treatment was obtained by covering the trees of the pistil donors in the field with a polyethylene cage (metallic structure covered with 0.178 mm polyethylene). The control trees were under the same metallic structure just covered with an insect proof to avoid bee pollination. This procedure revealed to be efficient to increase temperature without affecting other parameters (Rodrigo and Herrero, 2002a). The experiments were carried out over two years, one and three trees per treatment were used in the first and second year of the experiments, respectively. Temperature was monitored every 5 min outside (control treatment) and inside (warm treatment) the plastic cage with a 'data logger' (Testostor 175-3, Testo, Lenzkirch, Germany) placed at 60 cm above the soil surface, protected from the sun and oriented to the north. The plastic cage was maintained for up to two weeks in both years.

Pollination techniques

Two different crosses were carried out: 'Vignola' x 'Napoleon' in the first year and 'Sunburst' x 'Burlat' in the second year. To avoid the potential interference of flower emasculation on fruit set (Hedhly, 2003), evaluation was carried out in non-emasculated flowers. Several branches were randomly chosen from both the control and warm treatments the day prior to anthesis and, to have a synchronized population of flowers of the same age, only flowers at balloon stage (Baggiolini, 1952) were left in the tree, whereas all the others were removed. After this process all the selected branches presented at least 80 flowers/branch. This flower population was distributed randomly between treatments with a minimum of 250 flowers/treatment. In the second year, a second experiment was performed with flowers treated similarly to evaluate the reproductive process and a number of reproductive biology parameters (see below). To obtain pollen, flowers from the pollen donors were collected at balloon stage from 6 trees/pollen donor; anthers were separated from

their filaments and left to dehisce on a piece of paper at room temperature during 24-48 h. Pollen was then sieved through a 0.26 μm mesh, and frozen at –20ºC. Pollination was carried out on the day of flower anthesis and prior to anther dehiscence to avoid self pollination.

Evaluation of the onset of fruiting and flower and fruit drop

Flower and fruit drop were monitored in the two experiments, the control and the warm treatments, by weekly counts from anthesis to fruit ripening. With the aim of establishing the times of more intense dropping, we calculated the relative drop as the percentage of flower/fruit dropped each week in relation to the initial number of flowers at anthesis. Proportion comparisons were analyzed using Yates' Chi-square Goodness of Fit Test in 2x2 contingency tables testing for association between temperature and both initial and final fruit set as well as relative fruit drop.

Microscopic preparations

In the second year of the experiment, a batch of 10 flowers/treatment was daily fixed in FAA (formalin: acetic acid: 70% ethanol, 1: 1: 18 v/v; Johansen, 1940) during 10 days after anthesis from the 'Sunburst' X 'Burlat' cross. Microscopic observations were made of squashed stigma-styles washed three times in water, one hour each, autoclaved for 10 min at 1 kg/cm2 in 5% sodium sulfite (Jefferies and Blecher, 1974), and stained with 0.1% aniline blue in 0.1 N K3PO4 (Linskens and Esser, 1957). The ovaries were separated in the washing step and maintained in distilled water overnight. The next day, the ovary area was measured under a binocular microscope (Wild M8) and using an image analysis system (Quantiment 570, Leica, Cambridge, UK) connected to a camera (Cohu 8310 RGB colour camera, San Diego, Calif., USA), Then, the obturators and ovules were dissected under the binocular microscope, and the ovule area measured before squashing and staining with aniline blue. Preparations were examined under an Ortholux II microscope equipped with UV epifluorescence with a band pass 355-425 exciter filter and an LP 460 barrier filter. Ovules were scored for pollen tube penetration and also for ovule viability through the presence of callose deposits in the chalaza of degenerating ovules (Anvari and Stösser, 1978).

Proportion comparisons were analyzed using Yates' Chi-square Goodness of Fit Test in 2x2 contingency tables testing for association between temperature and ovule viability. For the percentage of flowers with pollen tube at style base, Fisher's exact test was used. Analyses of variance were carried out for ovule and ovary growth, pollen tube growth along the style and

the number of pollen tubes at the stylar base. The SPSS package (v 11.0.1, SPSS Inc., Chicago, Illinois) was used for all analysis.

Results

Fruit set

The plastic cage induced an appreciable increase in temperature (Table 1). While the average minimum temperature was practically unaffected by the plastic cage, the average maximum temperature increased 5.8ºC the first year and 6.9ºC the second year. This resulted in an average increase in the mean temperature of 1.4ºC and 3ºC, respectively. This moderate increase in temperature drastically reduced fruit set in both experiments (Figure 1a and b), from 18% to 5% in 'Vignola' and from 25% to 4% in 'Sunburst'. In both cultivars, a highly significant effect of temperature was revealed for the initial 4 weeks after anthesis ('Vignola' N = 533, Yates' χ^2= 27.870, $p < 0.000$; 'Sunburst': N = 452, Yates' $\chi^2 = 50.533$, $p < 0.000$), and final fruit set ('Vignola' N = 533, Yates' $\chi^2 = 20.743$, $p < 0.000$; 'Sunburst': N = 452, Yates' $\chi^2 = 40.862$, $p < 0.000$).

The analysis of flower/fruit drop revealed that the final fruit set was established during the first three to four weeks following pollination. Chi-square analysis revealed no significant association between temperature and fruit set after the forth week in both cultivars ('Vignola' N = 92, Yates' $\chi^2 = 0.161$, $p = 0.688$; 'Sunburst': N = 76, Yates' $\chi^2 = 0.011$, $p = 0.91$). Indeed, flower drop in both conditions was concentrated between the second and the fourth week (Fig. 1c and d), and in the warm treatment this flower drop was significantly higher during the third week for both years (1999: N = 497, Yates' $\chi^2 = 52.411$, $p < 0.000$; 2001: N = 391, Yates' $\chi^2 = 22.624$, $p < 0.000$). These results indicate that the effect of temperature occurred before the third week following anthesis.

The onset of fruiting

The comparison of ovary growth in the warm and control treatments shows similar growth patterns. Although the process appeared somehow accelerated under the warm treatment, no significant effect was found for the nine days following anthesis (Figure 2). Ovary growth started five days after pollination and was apparent by 7 days after pollination. To evaluate if this ovary growth was related to ovule growth, ovule area was also measured. In cherry, from the two ovules present in an ovary, usually only one, the primary ovule, develops into a seed; the other, the secondary ovule usually aborts. Sequential examination of the ovules showed

that, indeed, the secondary ovule did not grow and remained with a stable area of 0.48 mm^2 and eventually shriveled. On the contrary, the primary ovule started to grow 4 days after pollination and growth was most conspicuous 7 days after pollination. Thus, ovule growth encompassed ovary growth. As for ovary growth, temperature did not affect significantly ovule growth. Interestingly, for both parameters, growth did not occur in all ovaries or ovules and a population of flowers, presumably those that will eventually drop, did not continue growing. The separation of these two populations of flowers occurred as early as seven days after pollination suggesting that the reproductive phase might be the main candidate for the effect of temperature.

The reproductive phase

To evaluate the events that occurred within this frame of time, the progamic phase, spanning from pollination to fertilization (Linskens, 1986), was characterized under field conditions. Pollen germinated within 24 hours after pollination (Fig 3a), and penetrated the transmitting tissue in the style (Fig 3b) reaching the base of the style 2 to 4 days after pollination (Fig 3c). Within the ovary, the pollen tubes grew on the surface of the obturator to reach the ovule. By this time the secondary ovule had already started degeneration, shown by the deposition of callose at the chalaza that spreads over the nucellus and integuments (Fig 3d). The pollen tubes entered the micropyle and traversed the nucellus to achieve fertilization (Fig 3e) 6 days after pollination and the first embryos were soon apparent (Fig 3f).

This process was also studied in the warm treatment. Temperature accelerated significantly pollen tube growth only during the first days after pollination ($F = 5.888$, $p = 0.026$) (Figure 4a). The number of pollen tubes reaching the stylar base slightly increased during the first days after pollination but, at higher temperatures, a significant lower number of pollen tubes were recorded once the process was established (Figure 4c). Thus, while an average of 3.7 pollen tubes per flower was observed in the control treatment, only 2.0 pollen tubes were recorded in the warm treatment. ANOVA analysis on pooled data over the six last days, when the number of pollen tubes at style base stabilized and normally no further pollen tube growth takes place in the style, revealed a significant reduction in the number of pollen tubes at the base of the style in the warm treatment ($F = 6.543$, $p = 0.012$)

In the ovary, the first pollen tubes on the obturator were observed in the control treatment 3 days after pollination, but the warm treatment advanced this step by one day. Most flowers had pollen tubes at this structure 4 days after pollination in both treatments. But the proportion of pistils showing pollen tubes in the obturator was 68% in the warm treatment

compared to 88% in the control. This proportion was reduced again along the rest of the ovary pathway and only 12% and 25% of the flowers got fertilized in the warm and the control treatments, respectively. Interestingly, the fertilization level registered in the control corresponded to the final fruit set registered in the same experiment.

Ovule degeneration

To evaluate to which extent ovule degeneration may be responsible for these reduced fertilization levels, ovule viability has been evaluated in the same samples (Table 2). Degeneration of the secondary ovule was already apparent in 30 % of the flowers at anthesis and occurred in 100 % of the flowers after 3 days in the warm treatment and 4 days in the control, although one day after anthesis 70% of the flowers had the secondary ovule degenerated in both treatments. Also, in both conditions, 20 % of the flowers presented both ovules degenerated at anthesis. While the warm treatment accelerated the degeneration of both first and secondary ovules, the final percentage of flowers with two degenerated ovules was unexpectedly the same (60%) under both conditions. Yates' chi-square test revealed no significant effect of temperature on ovule viability over the pooled data through the 5-10 days after anthesis. Thus, around 40% of flowers presented at least one viable ovule, which represents the theoretical maximum fertilization rate that could be achieved.

Discussion

A small increase in temperature at flowering time drastically reduced fruit set in two different weet cherry cultivars and in two different years. While the increase in mean temperature was low, 1.4ºC and 3ºC, and the average minimum temperatures were similar in both treatments, an average increase of 5-7ºC in the maximum temperatures was registered, which could potentially explain this effect. A negative effect of high temperatures at flowering on the subsequent fruit set has been reported in other fruit tree species as apricot (Burgos et al., 1991), olive (Cuevas et al., 1994), and peach (Kozai et al., 2004). The analysis of the relative flower/fruit drop revealed that final fruit set is defined during the first 3-4 weeks following anthesis. This time period agrees with the time recorded for sour cherry (Bradbury, 1929; Lech and Tylus, 1983), apricot (Rodrigo and Herrero, 2002b) and other fruit trees (Sedgley and Griffin, 1989). Moreover, ovary and ovule growth shows that around seven days after pollination, a population of flowers starts to grow, while others remain unchanged and eventually drop. This timing points to the progamic phase as the main vulnerable process to warm temperatures.

An effect of temperature on the reproductive phase has been observed. Subjecting pollinated flowers to higher temperatures accelerated pollen tube growth rate in the pistil. However, the number of pollen tubes reaching the stylar base was reduced. The accelerating effect of higher temperatures on pollen tube growth was first reported in Oenothera (Lewis, 1942) and appears to be a general feature in different plant species. It has been widely reported in fruit trees (pear: Mellenthin et al., 1972; Lombard et al., 1972; almond: Socías i Company et al., 1976; plum: Jefferies et al., 1982; apple: Williams et al., 1984; sour cherry: Cerovic and Ruzic, 1992a; apricot: Austin et al., 1998; sweet cherry: Hedhly et al., 2004; peach: Hedhly et al., 2005b), and in herbaceous plants (ryegrass: Elgersma et al., 1989; alfalfa: Katepa-Mupondwa et al., 1996; groundnut: Kakani et al., 2002). However, few works have dealt with pollen tube dynamics expressed as the variation in the census of pollen tubes in the style. The results obtained in this work in the field agree with recent findings, under laboratory conditions, reporting a negative effect of high temperatures on pollen dynamics of several sweet cherry cultivars (Hedhly et al., 2005a) although in some peach cultivars a positive effect was registered (Hedhly et al., 2005b). These discrepancies could reflect the different adaptation of these two species to temperature since sweet cherry is adapted to regions with lower temperatures than peach. Furthermore, within a given species, genotypic differences were recorded in this parameter reflecting the adaptation to temperature of the pollen donor (Hedhly et al., 2004).

The occurrence of degenerated ovules early at anthesis registered in this work seems to be a common feature in sweet and sour cherry (Eaton, 1959; Furukawa and Bukovac, 1989; Thompson, 1996; Cerovic and Micic, 1999). Anomalies in ovule and embryo sac development have also been registered in other fruit trees such as apricot (Eaton and Jamon, 1965), pistachio (Grundwag and Fahn, 1969), avocado (Tomer and Gottreich, 1976), olive (Rallo et al., 1981), or almond (Pimienta and Polito, 1983), and these anomalies have been related to fruit set. In sweet cherry, it has been put forward that short ovule and embryo sac longevity could potentially explain the level of fruit set obtained (Eaton, 1959), mainly when a delay in pollination occurs (Stösser and Anvari, 1982, 1983). Likewise, ovules are considered to be highly vulnerable structures to increasing temperatures (Williams, 1970, Stösser and Anvari, 1982; Postweiler et al., 1985, Cerovic and Ruzic, 1992b, Cerovic et al., 2000). While at anthesis both ovules were degenerated in a small proportion of the flowers, this proportion increased to 60% at fertilization time. In this work, although high temperature accelerated ovule degeneration, surprisingly the final percentage of flowers with at least one viable ovule, or with both ovules degenerated, was similar in the warm and control

treatments. Early ovule degeneration accounts in part for reduced fruit set, but cannot fully explain the percentage of fertilized ovules, since in spite of having 40 % of the flowers with viable ovules at the time of fertilization only 25% got fertilized in the control, which correspond to the final fruit set. In the warm treatment this proportion was reduced to 12% that yielded a 4% final fruit set. Thus, warm temperature could also affect pollen tube growth in the ovary in this species. Differences between fertilization rate and actual fruit set in the warm treatment could be, however, also explained by an effect on early embryo development and/or by fruit drop.

The experimental procedure used herein applying the warm treatment specifically at flowering time together to the results obtained along the reproductive phase underline the consequences that slight increases in temperature at this time may have on the subsequent fruit set. The results obtained in this work together to those previously obtained on the shortening of stigmatic receptivity with increasing temperatures (Hedhly et al., 2003) show that an increase in the mean temperature as low as 1.4 to 3ºC during the first 2 weeks after anthesis has a direct effect on fruit set and could explain the erratic bearing recorded in some years with apparent "good" and warm springs. Due to climate change, these situations of warm springs could be more frequent in the future; in fact, the evaluation of temperature increase in time series shows that increases are most conspicuous at this time of the year (Easterling et al., 1997, Sparks and Menzel, 2002). Anyhow these temperature increases can be common in Mediterranean areas, and the results obtained in this work will contribute to explain the erratic fruit set reports registered in this and other temperate fruit tree species.

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Figure Legends:

Fig. 1: Effect of temperature on flower/fruits drop pattern (a, b) and times of maximum flower/fruit drop (c, d) under control and warm conditions in the crosses ’Vignola’ x ‘Napoleon’ (a, c) and ‘Sunburst’ x ‘Burlat’ (b, d).

Fig. 2: Ovary and ovule growth following pollination under control and warm conditions in the cross 'Sunburst' x 'Burlat'.

Fig. 3: The reproductive process. (A) Pollen grains germinate and traverse the stigmatic surface within 24 hours after pollination. Bar = 70 μm. (B) Pollen tube growth along the transmitting tissue 2 days after pollination. Bar = 200 μm. (C) One pollen tube at the base of style 3 days after pollination. Bar = 20 μm. (D) A degenerated ovule: callose deposits located in the chalaza spread over the nucellus and some parts of integuments 4 days after pollination. Bar = 150 μm. (E) Pollen tube penetrating the nucellus to carry out embryo sac fertilization 6 days after pollination. Bar = 70 μm. (F) Fertilised ovule showing a young embryo 8 days after pollination. Bar = 70 μm.

Fig. 4: Pollen tube growth in the style up to ten days after pollination in the cross 'Sunburst' x 'Burlat' under control and warm conditions expressed as (a) the percentage of style traveled by the longest pollen tube, (b) the percentage of flowers with pollen tubes at the stylar base, and (c) the number of pollen tubes at the stylar base. Vertical bars represent standard errors.

Fig. 1

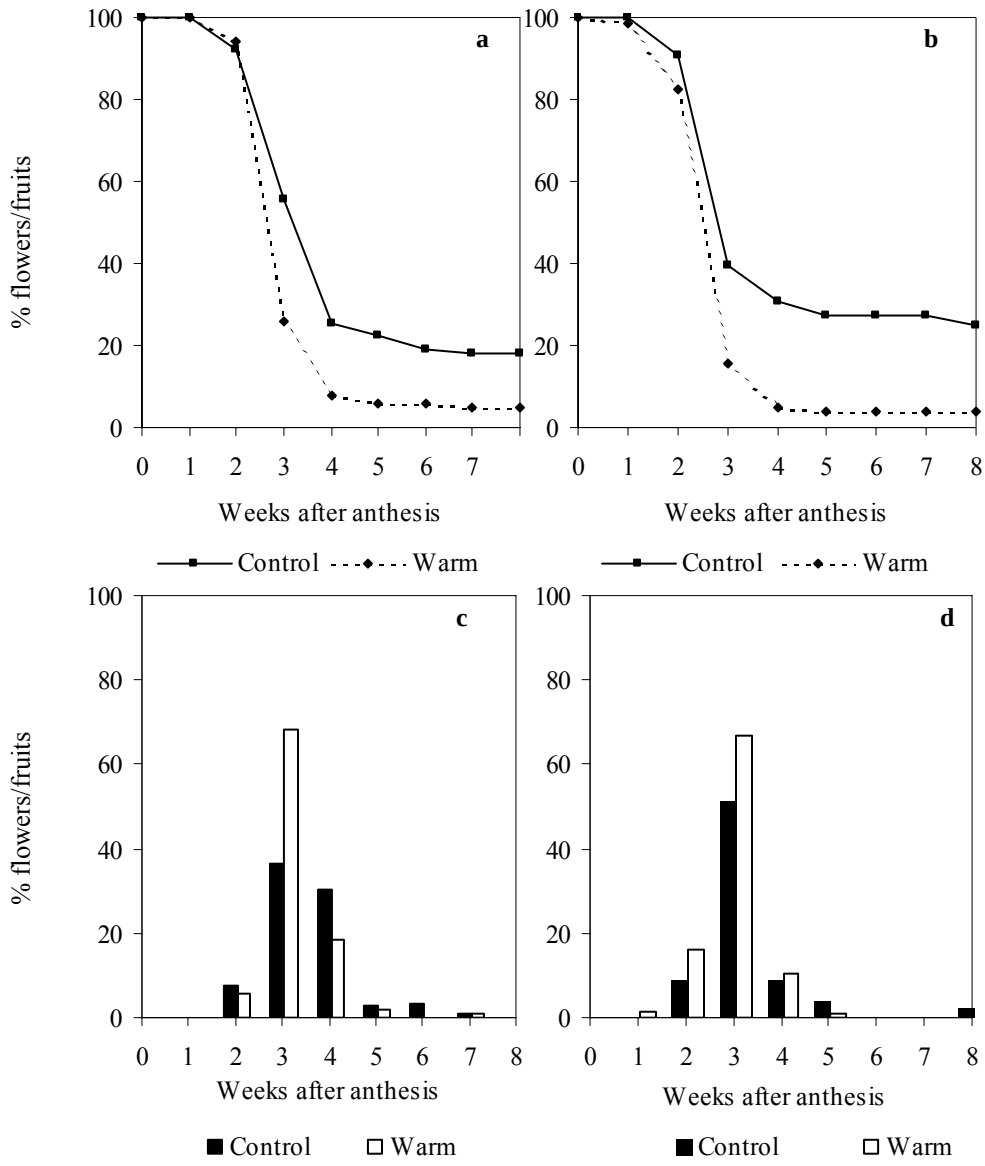


Fig. 2

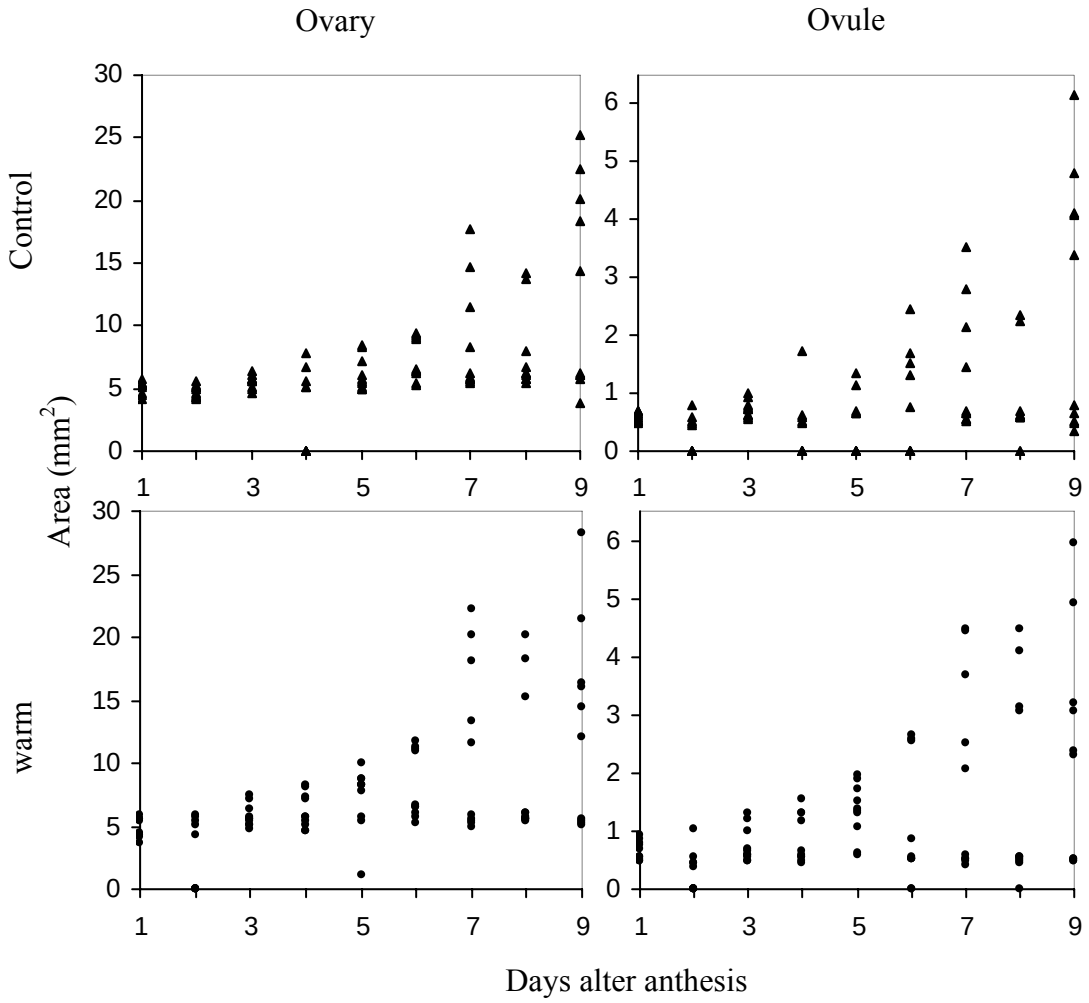


Fig. 3

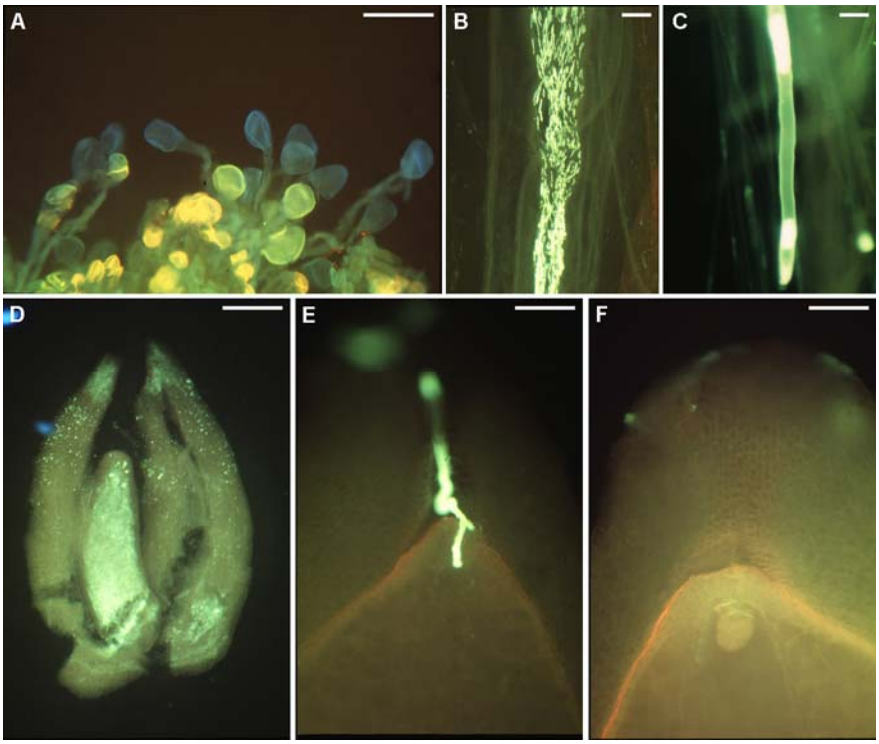
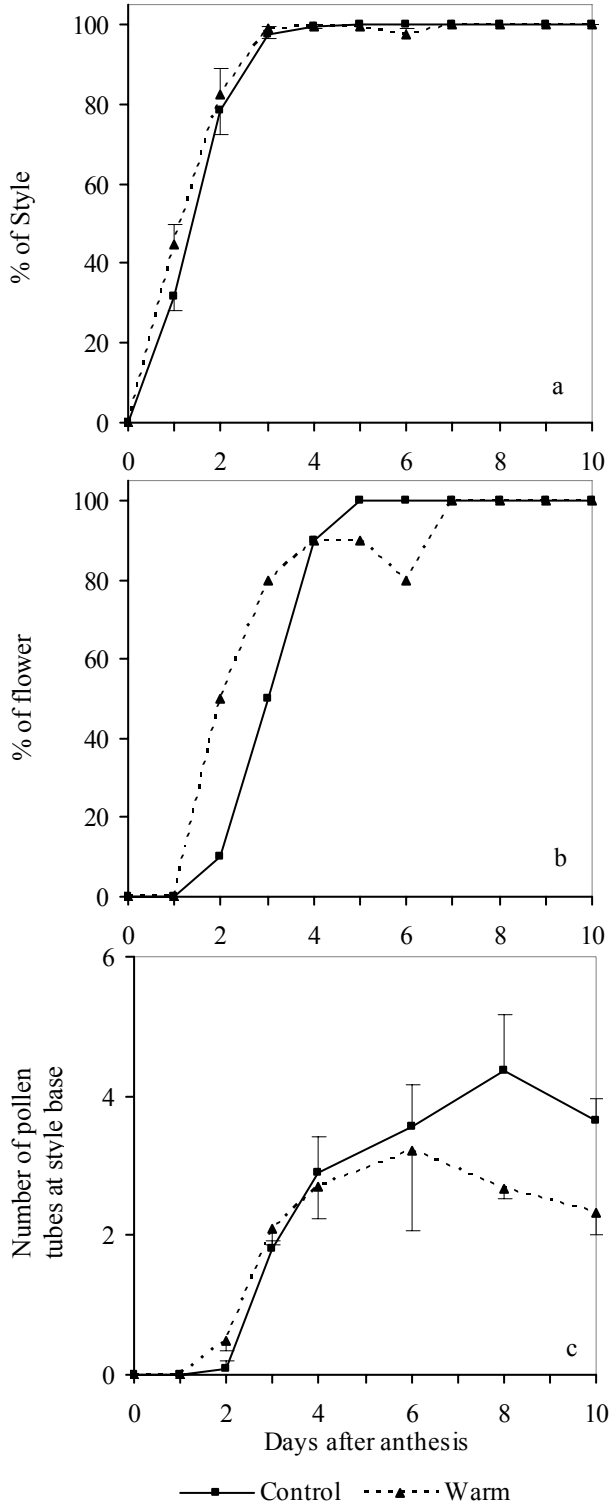


Fig. 4


% of Style
100
80
60
40
20
0
a
0
2
4
6
8
10
% of flower
b
Number of pollen tubes at style base
6
4
2
c
Days after anthesis
Control
Warm

Tab. 1: Average temperatures (ºC) registered in the control and warm treatments for two weeks following pollination in the two years of experiments.

	Maximum		Minimum		Average	
	Control	Warm	Control	Warm	Control	Warm
First year	23.7	29.5	7.6	6.7	14.7	16.1
Second year	24.1	31.0	7.3	8.4	15.2	18.2

Tab. 2: Effect of temperature on ovule longevity expressed as the percentage of flowers with one (secondary), and two (primary and secondary) degenerated ovules under warm and control conditions in the cross 'Sunburst' x 'Burlat'.

		Days after anthesis					
		0	1	2	3	4	Average 5-10
Flowers with one degenerated ovule (%)	Control	30	70	60	70	100	100
	Warm	30	70	90	100	100	100
Flowers with two degenerated ovules (%)	Control	20	10	20	20	60	**60**
	Warm	20	20	30	60	60	**59**